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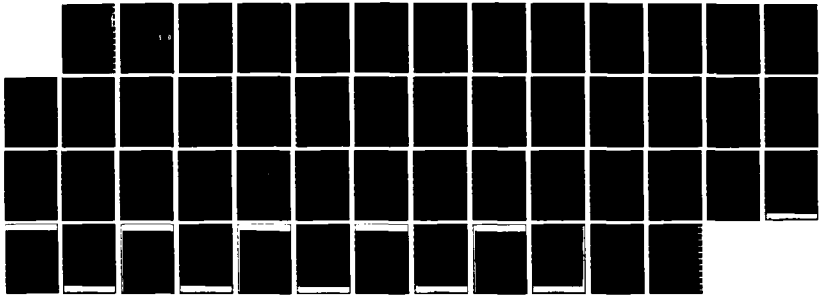

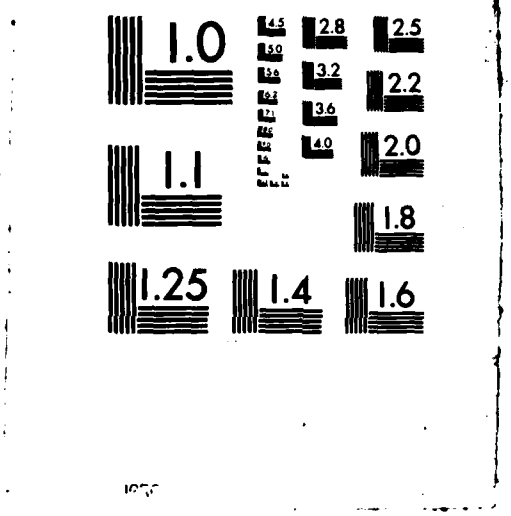
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THE USE OF HYPERTONIC SOLUTIONS TO RESUSCITATE ANIMALS FROM HYPOVOLEMIC SHOCK

ANNUAL REPORT

James W. Holcroft, M.D.

George Kramer, Ph.D.

Jerry F. Green, Ph.D.

August 31, 1984

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-83-C-3175

University of California, Davis

Davis, California 95616

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) THE USE OF HYPERTONIC SOLUTIONS TO RESUSCITATE ANIMALS FROM HYPOVOLEMIC SHOCK		5. TYPE OF REPORT & PERIOD COVERED ANNUAL REPORT 07/01/83 - 06/30/84
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) James W. Holcroft, M.D. George Kramer, Ph.D. Jerry F. Green, Ph.D.		8. CONTRACT OR GRANT NUMBER(s) DAMD17-83-C-3175
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of California, Davis School of Medicine Davis, CA 95616		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Program Element-62772A Project-3S162772A874 Task Area-AB Work Unit-261
11. CONTROLLING OFFICE NAME AND ADDRESS U. S. Army Medical Research & Development Command Fort Detrick, Frederick, Maryland 21701		12. REPORT DATE August, 1984
		13. NUMBER OF PAGES 41
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release;
distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Hypertonic sodium chloride
Human beings
Trauma
Hemorrhagic shock
Shock
Major surgery

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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FIGURE 2

ABSTRACT

For the past year we have studied the use of hypertonic solutions to resuscitate animals from hypovolemic shock. In brief, it appears that a solution comprised of sodium chloride, with a calculated osmolality of 2,400 milliosmols/kg mixed with a dextran solution, provides good resuscitation from hemorrhagic shock in sheep. This resuscitation is longer lasting than resuscitation with equivalent volumes of normal saline. The resuscitation also seems better than when using solutions containing sodium acetate, mannitol, glucose, or sodium bicarbonate.

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SUMMARY

For the past year we have studied the use of hypertonic solutions to resuscitate animals from hypovolemic shock. In brief, it appears that a solution comprised of sodium chloride, with a calculated osmolality of 2,400 milliosmols/kg mixed with a dextran solution, provides good resuscitation from hemorrhagic shock in sheep. This resuscitation is longer lasting than resuscitation with equivalent volumes of normal saline. The resuscitation also seems better than when using solutions containing sodium acetate, mannitol, glucose, or sodium bicarbonate.

FOREWORD

Hypertonic solutions have been used since the early 1900's to resuscitate patients from shock. They are frequently used now, and have been used for some several years, to resuscitate patients from burn shock (2, 4, 13, 19). They seem to achieve adequate cardiovascular resuscitation with less volume than isotonic solutions.

Extremely hypertonic solutions have been used to resuscitate dogs, sheep, rats, and pigs from hypovolemic shock (1, 3, 8, 14, 15, 21). These solutions achieve excellent cardiovascular resuscitation and do so with infusion of very small quantities of fluid. In addition, in dogs (21) and pigs (personal communication, Dr. L. William Traverso, Letterman Army Institute of Research), the extremely hypertonic solutions seem to improve survival rates when compared to infusion of equivalent volumes of normal saline.

Shackford and his colleagues in San Diego, in a controlled, prospective, randomized, clinical trial, studied the effects of infusing hypertonic solutions to patients undergoing reconstructive surgery on the infrarenal abdominal aorta (18). Fifty-eight patients were entered into a study that lasted 22 months. Twenty-eight patients received, along with blood products, Ringer's lactate to maintain hemodynamic stability; 30 patients received a hypertonic balanced salt solution. This solution was made so that its sodium concentration was 250 mEq/l, its lactate concentration 128 mEq/l, its chloride concentration 129 mEq/l, its potassium concentration 4 mEq/l, and its calcium concentration 3 mEq/l. Its calculated osmolarity was 514 milliosmol/l. The two groups were similar in their baseline characteristics.

The Ringer's lactate group required 10 liters of fluid for maintenance of cardiovascular stability; the hypertonic group, 5 liters. The patients in the Ringer's lactate group, on the first postoperative day, were eight kg heavier than their baseline weight; the patients in the hypertonic group were four kg heavier. This difference in weight gain between the two groups persisted for the first three postoperative days. Pulmonary function, after fluid administration, was slightly better in the hypertonic group: the shunt fraction in the Ringer's lactate group was 20% compared to 16% in the hypertonic group.

We became interested in the use of hypertonic solutions to resuscitate animals from shock based on this clinical experience and based on experience with animals that is described in the initial contract proposal.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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BODY OF REPORT

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The results of evaluating different hypertonic solutions are indicated in the abstract that is reproduced on the following page. Small volumes of hypertonic sodium chloride solutions and small volumes of other solutions were used to resuscitate animals that have been subjected to a moderate degree of hemorrhagic shock. The sodium chloride-dextran solution restored cardiovascular measurements closest to normal and maintained those values at close to normal levels for about 30 minutes after resuscitation.

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HYPERTONIC RESUSCITATION: A COMPARISON OF THREE SOLUTIONS

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Small volumes (4 ml/kg) of 2400 mOsm NaCl restore cardiac output and mean arterial pressure to 80% of baseline after hemorrhage (65% of blood volume) in unanesthetized sheep. An equal volume of normal saline is less effective. To identify an optimal hypertonic solution, we screened six 2400 mOsm solutions in 18 randomized experiments in 8 sheep: NaCl, NaCl/sodium acetate, NaCl/6% Dextran 70, $NaHCO_3$, glucose, and mannitol/NaCl. Cardiovascular function was restored best with NaCl, NaCl/NaAc, and NaCl/Dex which were then evaluated using 17 sheep in 35 experiments. Following a 1 hour baseline period, the sheep were bled to a mean arterial pressure of 50 mmHg for 2 hours. One of the solutions was then given in a volume of 4 ml/kg over 2 minutes. All solutions were given in a blinded and randomized order.

Cardiac Output, l/min, ± SD

Solution	(n)	Baseline	End of Hemorrhage	Post Infusion 10'	Post Infusion 180'
NaCl	(12)	4.9 ± .7	2.2 ± .5	4.4 ± .7	3.9 ± 0.7
NaCl/NaAc	(11)	5.1 ± .9	2.2 ± .7	4.7 ± .9	3.8 ± 1.0
NaCl/Dex	(12)	5.2 ± .9	2.1 ± .5	5.3 ± .6*	4.6 ± 0.7*

Plasma Volume, ml/kg, ± SD

NaCl	(10)	45 ± 4.6	30 ± 4.9	41 ± 6.6	34 ± 7.0
NaCl/NaAc	(9)	43 ± 5.1	29 ± 2.9	38 ± 3.1	33 ± 5.6
NaCl/Dex	(11)	46 ± 4.8	30 ± 5.3	44 ± 5.7	41 ± 5.4*

*($p < .05$ by ANOVA and Bonferroni's corrected t test)

Serum osmolality increased 10% and serum sodium concentrations increased from 145 to 155 mEq/l. In conclusion, all three solutions restored cardiac output to 85% of baseline, rapidly though transiently. The NaCl/Dex solution sustained this effect better than the other solutions, perhaps by maintaining plasma volume.

Infusion of very hypertonic saline to bled rats: Membrane potentials and fluid shifts.

Running title: Hypertonic saline and shock.

Key words:

hypertonic saline

hemorrhagic shock

hypotension

intracellular water, sodium, chloride

membrane potential

rat resuscitation

skeletal muscle

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Abstract

We subjected anesthetized rats to a moderate degree of hemorrhagic shock, lowering their mean arterial pressure to approximately 50 mmHg for approximately 100 minutes. At the end of the shock period, resting skeletal muscle transmembrane potentials had depolarized from a baseline value of -82 mv to -65 mv; intracellular water had increased by 13%; and intracellular sodium and chloride contents had doubled. Eight rats were then given an infusion of very hypertonic saline (2400 mOsm/kg, calculated osmolality) in a volume equal to only 10% of the volume of shed blood; another 8 rats were given the equivalent amount of sodium and chloride in an isotonic solution (volume equal to 80% of shed blood). The mean arterial pressure in the rats that were given the very hypertonic saline returned to 81 mmHg, compared to 55 mmHg in the animals given normal saline. The membrane potentials in the hypertonic group polarized back to near normal -- -78 mv -- compared to no changes in the normal saline group. Intracellular water returned to preshock values in the hypertonic group as did intracellular sodium and chloride contents. Cellular contents in the normal saline group remained at shock levels. We conclude that, in rats, infusion of small amounts of hypertonic saline can reverse some of the cellular abnormalities induced by hemorrhagic shock.

Introduction

Water shifts into the cells of skeletal muscle during hemorrhagic shock(5). Administration of hypertonic saline (1,4,6) might be expected to draw some of this water out of these swollen cells. We examined the effects of administering very small quantities of very hypertonic saline (2400 mOsm/kg) on skeletal muscle water in anethetized rats that had been subjected to hemorrhagic shock. We also examined the effects of the hypertonic saline on intracellular sodium and chloride contents and on skeletal muscle resting transmembrane potentials. These effects were compared with those induced by infusing an equivalent amount of solute administered as normal saline.

METHODS

Wistar rats (257 to 484 g) were allowed free access to food and water until one hour before being anesthetized with chloralose-urethane (3%-4%, 2 ml/kg, intraperitoneally). They were then restrained in the supine position on a heating pad that was thermostatically controlled to maintain a rectal temperature of 37 C. The right femoral artery and vein were cannulated with polyethylene tubing, the ends of the catheters being placed approximately in the abdominal aorta and inferior vena cava. Heart rate was recorded and blood pressure was monitored with a pressure transducer. Resting skeletal muscle transmembrane potentials were measured with a microelectrode in individual cells of the femoral muscles after exposing the muscles by incising the overlying skin. A 30 mg piece of biceps or pectoralis major muscle was excised for determination of muscle water and electrolytes. One half ml of blood was drawn into a syringe that was coated with sodium heparin for measurement of hematocrit and plasma sodium, potassium, and chloride concentrations.

Blood was then withdrawn out of the venous catheter until the animal's mean arterial pressure fell to 50 mmHg, the hemorrhage lasting approximately 10 minutes. The shed blood was anticoagulated with sodium heparin and stored at room temperature. Arterial blood pressure was maintained at 50 mmHg for 90 minutes by further hemorrhage as required. At the end of this shock period, membrane potentials were measured, a muscle biopsy was obtained, and blood was drawn for hematocrit and electrolyte

determinations, the blood being replaced volume for volume by the previously shed blood. These procedures took approximately 15 minutes.

The animals were then entered into one of three groups: (1) 8 animals were resuscitated with very small quantities (volume = 10% of shed blood) of very hypertonic saline (2400 mOsm/kg, calculated osmolality); (2) 8 animals were resuscitated with larger quantities (volume = 80% of shed blood) of normal saline (308 mOsm/kg, calculated osmolality); (3) 6 animals were followed without resuscitation. The resuscitated fluids were infused through the femoral venous catheter. The hypertonic saline was infused over a period of one minute; the normal saline over two minutes. No other resuscitative measures were undertaken: no blood was given, except to replace blood that was withdrawn for measurement of hematocrit and electrolytes; no maintenance fluids were given.

Thirty minutes after infusion of the hypertonic saline or normal saline, membrane potentials were measured, a muscle biopsy was obtained, and blood for hematocrit and electrolytes was withdrawn. Similar measurements were made between 60 and 90 minutes after infusion of the fluids.

Resting skeletal muscle potentials were measured with a Ling-Gerard microelectrode (5). Potentials were recorded in at least 25 cells; these values were averaged to give the resting potential. Biopsies of skeletal muscle were taken from deep within the muscle mass. One part of the biopsy was dried to constant weight at 75 C to determine total water content. Two

hundred microliters of Triton X detergent was added to a second part. The biopsy-detergent mixture was agitated at room temperature for 24 hours and then heated to 80 C in a water bath for 24 hours. The resultant suspension was centrifuged to produce a clear supernatant. Sodium and potassium concentrations in the supernatant, and in plasma, were determined by flame photometry; chloride concentrations were determined by a Digital Chloridometer. All determinations were performed in duplicate. Intracellular and extracellular water and electrolytes were calculated as described previously (2).

Results in the tables and text are expressed as means $\pm$ 1 standard deviation (SD) to describe distributions of the measurements. Results in the figures are displayed as means $\pm$ standard error of the mean (SE) to facilitate comparison of the two treatment regimens. A one way analysis of variance over time was used to determine if infusion of a solution was associated with a significant change in a variable. When such associations were demonstrated, a two-tailed t-test with the Bonferroni correction for multiple comparisons was used to test differences between the two treatment groups. Significance was accepted at the 5% level.

All experiments were conducted in accordance with institutional standards for care of laboratory animals.

Results

A representative experiment in a 409 g rat is indicated in Figure 1. During the baseline period, the mean resting skeletal muscle membrane potential was -84 mv. Just before time zero, blood and muscle were obtained for measurement of hematocrit, plasma electrolytes, muscle water, and muscle electrolytes. Starting at time zero and continuing for the next 90 minutes, 4.1 ml of blood was withdrawn to produce a mean arterial pressure of 50 mmHg. At 85 minutes the mean membrane potential had deteriorated to -60 mv. Blood and muscle were obtained. At 95 minutes, hypertonic saline, 2400 mOsm/kg, 0.4 ml, was infused over 1 minute. Blood pressure and potentials recovered over 30 minutes and then deteriorated over the next 90 minutes. Blood and muscle were obtained 25 and 80 minutes after infusion.

The animals in the three groups were subjected to the same degree of shock. The animals that were given hypertonic saline were bled slightly more than those given normal saline, but the animals in the hypertonic saline group were slightly heavier (Table 1). The animals in the normal saline group were in shock for several minutes longer than those in the hypertonic group (Table 1). By experimental design the blood pressures in the three groups at the end of the shock period were the same (Table 2). Deterioration of the membrane potentials were the same in all groups (Table 2).

The shock was moderately severe. Some animals had agonal respirations at the end of the shock period. Four of 6 rats

given no resuscitation had died by 210 minutes after induction of shock (Table 1). Mean arterial pressure, 60-90 minutes after the end of the shock period, in the animals given normal saline or no infusion, were at shock levels (Table 2).

During shock, sodium, chloride, and water moved into the cells in all groups (Table 3). Intracellular water content, expressed as ml/g dry weight of tissue, increased approximately 13%. Intracellular sodium and chloride content, at the end of the shock period, doubled compared to preshock values (Table 3). Sodium and chloride concentrations inside the cell also increased at the end of the shock period, compared to preshock values (Table 3).

Extracellular water decreased from a mean of 0.62 $\pm$ 0.14 (SD) to 0.36 $\pm$ 0.16 ml/g dry weight during shock. Concentrations of sodium, potassium, and chloride in the plasma did not change significantly. Thus, during shock, the extracellular content of these three ions all decreased to approximately 60% of baseline values.

Infusion of hypertonic saline transiently restored all measured abnormalities back toward normal. Blood pressure came back to within 20 mmHg of baseline values immediately after infusion and it then drifted down to shock levels (Figure 2). Similarly, membrane potentials, after infusion of hypertonic saline, came back to almost baseline values and then gradually deteriorated (Figure 3). Intracellular water returned to baseline values 30 minutes after infusion of hypertonic saline as did intracellular sodium and chloride contents (Figure 4); these values

remained close to baseline when measured 60 to 90 minutes after infusion (Figure 4).

Infusion of an equivalent amount of sodium and chloride as normal saline had little effect on blood pressure (Figure 2), membrane potentials (Figure 3), intracellular water (Figure 4), intracellular sodium content (Figure 4), and intracellular chloride content (Figure 4). These values all remained near those determined at the end of the shock period.

The differences in blood pressure, membrane potential, intracellular water, and intracellular sodium and chloride contents 30 minutes after infusion were all significantly different when the values in the animals given hypertonic saline were compared to the values in the animals given normal saline (Figure 4).

Discussion

The rats in all three groups were subjected to the same degree of shock. The shock was moderately severe: four of six non-resuscitated animals died within two hours of the end of the shock period; two of eight rats partially resuscitated with moderate amounts of normal saline died.

The hypertonic saline did not completely resuscitate the animals, but it was given as a single bolus and in extremely small quantities. Nonetheless it still rapidly corrected abnormalities of membrane potentials and of cellular sodium, chloride, and water contents, returning these values to normal within 30 minutes of infusion. The same amount of sodium and chloride, given as normal saline, had no beneficial effect on these cellular abnormalities.

Removal of excess cellular water by the hypertonic saline could be partly explained by hyperosmolar-induced shifts of water from the intracellular to the extracellular space. Correction of the other cellular abnormalities is more difficult to explain by the information obtained in these experiments. Even though difficult to explain however, the findings are striking--the skeletal muscle cells in the animals given very hypertonic saline were identical in their membrane potentials and ion contents to normal cells; skeletal muscle cells in animals given equivalent amounts of solute as normal saline and in animals given no resuscitation maintained their shock-like characteristics.

The methodology used in these experiments assumes that the

Donnan equilibrium holds even in the shock state. This assumption is accepted by most workers in the field for distribution of sodium and chloride between the vascular and interstitial spaces; some workers question, however, if the Donnan equilibrium remains unchanged during shock with respect to potassium (3). This question is impossible to answer at this time. All techniques of measuring interstitial potassium have methodologic weaknesses. Direct sampling of the interstitium by micropuncture, for example, can damage cells in the area of micropuncture; damage of only a few cells could increase interstitial potassium contents to values far higher than those actually present. In any case, the membrane potentials and the calculations of intra- and extracellular water, sodium, and chloride contents should be accurate; the calculations for intra-and extracellular potassium are the most open to question.

The weight of the animals varied, but this variation should not affect the results in any way. There are no reports in the literature that suggest that age or weight of an animal affects membrane potentials or cellular composition. In addition, during the baseline period, the membrane potentials and intracellular water, sodium, potassium, and chloride were the same in all three groups.

These studies should be taken as being strictly experimental. We do not recommend infusing very hypertonic saline to patients at this time, and we believe that much more work needs to be done on this subject before even preliminary trials in patients should be undertaken. At the same time it must be ad-

mitted that we have initiated these studies because of several features of very hypertonic saline that make it potentially attractive for use in patients. It seems to resuscitate animals rapidly, at least for 15 minutes or so, even when given in very small quantities, and thus might be a good solution to use in the initial resuscitation of patients subjected to trauma. The solution has a very low freezing point and is so hypertonic that bacteria cannot grow in it; thus the solutions could be stockpiled and kept for long periods of time to be used in cases of mass casualties. The solution is not viscous -- in contrast to other hypertonic solutions such as glucose -- and is easy to infuse. The solution also seems to have favorable hemodynamic effects, over and above those favorable effects that would be seen by plasma expansion alone.

In conclusion, the administration of very small amounts of very hypertonic saline to rats subjected to hemorrhagic shock rapidly restores membrane potentials and cellular water, sodium, and chloride contents to preshock levels. These responses are better than those seen by infusing an equivalent amount of solute in isotonic solution.

Acknowledgements

We gratefully acknowledge the excellent technical assistance of Teresa Keeler and Dave Shelby and also the able secretarial assistance of Lucille Bettanini.

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Legends for Tables

Table 1: Summary of experiments. Means $\pm$ 1 SD.

Table 2: Measured variables during hemorrhage and resucitation. * = significant difference ($p<0.05$) between groups given hypertonic and normal saline.

Table 3: Intracellular water, electrolyte contents, and electrolyte concentrations. See legend for Table 2.

	Hypertonic Saline	Normal Saline	No Resuscitation
Number of Animals	8	8	6
Body Wt. (g)	377 ± 58	332 ± 24	355 ± 64
Shed Blood (ml)	4.9 ± 1.1	3.7 ± 0.6	4.3 ± 1.0
Shock Period (min)	111 ± 15	118 ± 12	---
Survival at 210 min.	88% (7/8)	75% (6/8)	33% (2/6)

Table 1: Summary of Experiments. Means ± 1 SD.

		Baseline	Shock	After Infusion 30'	After Infusion 60'-90'
Arterial pressure (mmHg)	Hypertonic Saline	96 ± 12	49 ± 3	81 ± 7*	60 ± 10*
	Normal Saline	87 ± 13	46 ± 5	58 ± 6	47 ± 6
	No Infusion	91 ± 10	50 ± 5	47 ± 9	48 ± 16
Heart rate	Hypertonic Saline	362 ± 52	347 ± 59	395 ± 59	398 ± 101
	Normal Saline	350 ± 25	319 ± 33	334 ± 49	304 ± 54
	No Infusion	378 ± 32	353 ± 34	365 ± 56	440 ± 104
Hct (%)	Hypertonic Saline	50 ± 3	44 ± 4	41 ± 4	42 ± 3
	Normal Saline	49 ± 2	45 ± 3	41 ± 4	41 ± 4
	No Infusion	47 ± 3	40 ± 5	37 ± 3	43 ± 3
Membrane Potential (-mV)	Hypertonic Saline	83 ± 2	64 ± 5	78 ± 4*	74 ± 6*
	Normal Saline	82 ± 2	66 ± 3	63 ± 4	65 ± 6
	No Infusion	81 ± 2	66 ± 3	64 ± 4	62 ± 3

Table 2: Measured variables during hemorrhage and resuscitation. * = significant difference ($p<0.05$) between groups given hypertonic and normal saline.

		Baseline	Shock	After Infusion 30'	After Infusion 60'-90'
H_2O (ml/gDW)	Hypertonic Saline	2.3 ± 0.3	2.7 ± 0.2	2.3 ± 0.2*	2.4 ± 0.4
	Normal Saline	2.4 ± 0.2	2.7 ± 0.3	2.8 ± 0.2	2.7 ± 0.3
	No Infusion	2.4 ± 0.4	2.7 ± 0.4	2.5 ± 0.3	2.6 ± 0.2
Na^+ (mEq/100gDW)	Hypertonic Saline	2.4 ± 2.7	5.3 ± 2.8	1.4 ± 2.4*	2.5 ± 2.8
	Normal Saline	3.3 ± 2.3	5.3 ± 2.2	5.1 ± 2.3	5.1 ± 2.0
	No Infusion	0.9 ± 0.9	2.6 ± 2.2	2.4 ± 1.7	3.6 ± 1.1
K^+ (mEq/100gDW)	Hypertonic Saline	43 ± 5	46 ± 8	46 ± 7	45 ± 8
	Normal Saline	43 ± 6	50 ± 6	51 ± 5	46 ± 5
	No Infusion	45 ± 1	49 ± 3	48 ± 4	47 ± 2
Cl^- (mEq/100gDW)	Hypertonic Saline	1.2 ± 0.2	2.8 ± 0.5	1.4 ± 0.3*	1.9 ± 0.8
	Normal Saline	1.3 ± 0.2	2.6 ± 0.6	3.0 ± 0.5	2.9 ± 1.1
	No Infusion	1.3 ± 0.2	2.6 ± 0.7	2.5 ± 0.6	2.9 ± 0.3
$[Na^+]$ (mEq/l)	Hypertonic Saline	9.7 ± 11.8	19.8 ± 11.1	6.1 ± 10.5*	9.5 ± 10.0
	Normal Saline	13.9 ± 9.6	19.3 ± 7.7	17.5 ± 7.3	18.4 ± 6.0
	No Infusion	3.4 ± 3.3	8.8 ± 7.9	9.3 ± 6.4	13.8 ± 4.5
$[K^+]$ (mEq/l)	Hypertonic Saline	190 ± 16	174 ± 26	198 ± 24	190 ± 20
	Normal Saline	183 ± 32	185 ± 15	179 ± 13	170 ± 10
	No Infusion	194 ± 26	185 ± 26	197 ± 36	177 ± 17
$[Cl^-]$ (mEq/l)	Hypertonic Saline	5.0 ± 0.6	10.6 ± 2.1	6.1 ± 1.1*	7.6 ± 2.2
	Normal Saline	5.6 ± 0.4	9.6 ± 1.6	10.7 ± 1.6	10.4 ± 3.4
	No Infusion	5.4 ± 0.7	9.4 ± 1.1	10.0 ± 1.5	11.4 ± 1.4

Table 3: Intracellular water, electrolyte contents, and electrolyte concentrations. See legend for Table 2.

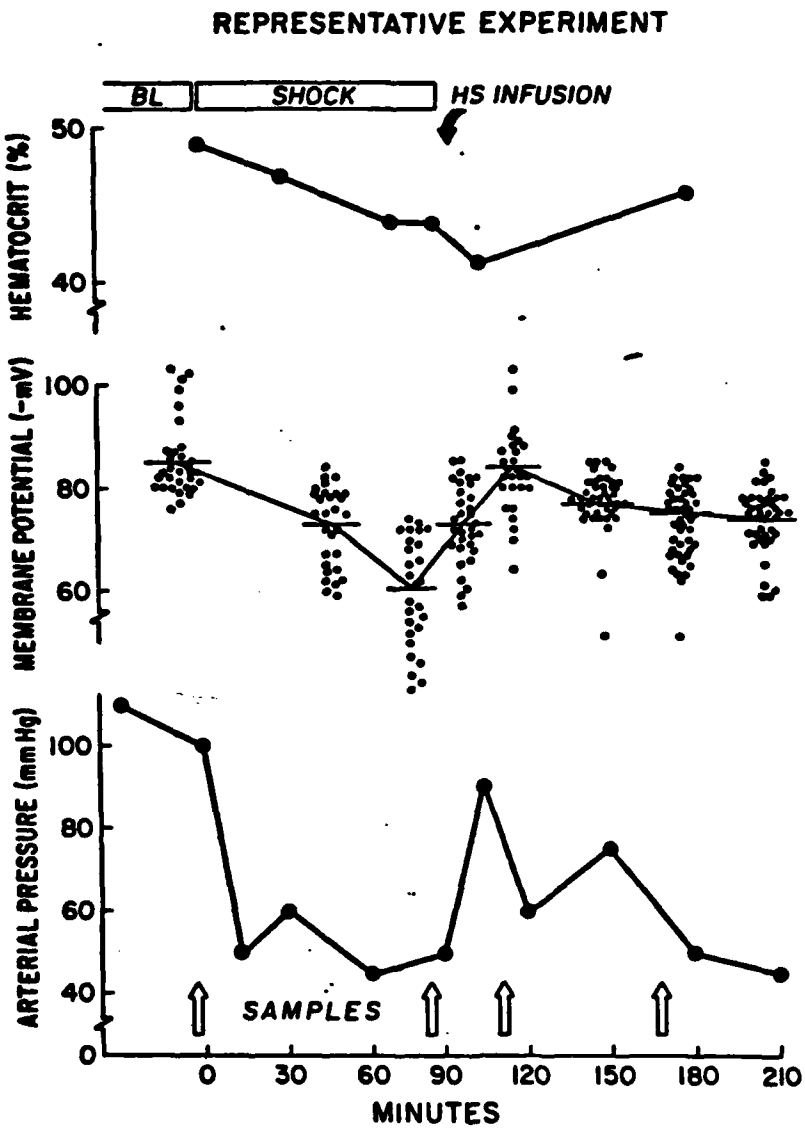
Legends for Figures

Figure 1: Representative experiment. Each point for membrane potentials represents measurement in a single cell; horizontal lines are means of all measurements. Open vertical arrows indicate sampling of blood and muscle. BL = baseline period; HS = hypertonic saline.

Figure 2: Mean arterial pressures in 8 rats given hypertonic saline (HS) and in 8 given same total solute load as normal saline (NS). * = significant difference ($p<0.05$) between groups.

Figure 3: Resting skeletal muscle membrane potentials. See legend for Figure 2.

Figure 4: Intracellular contents of water, sodium, potassium, and chloride. See legend for Figure 2.



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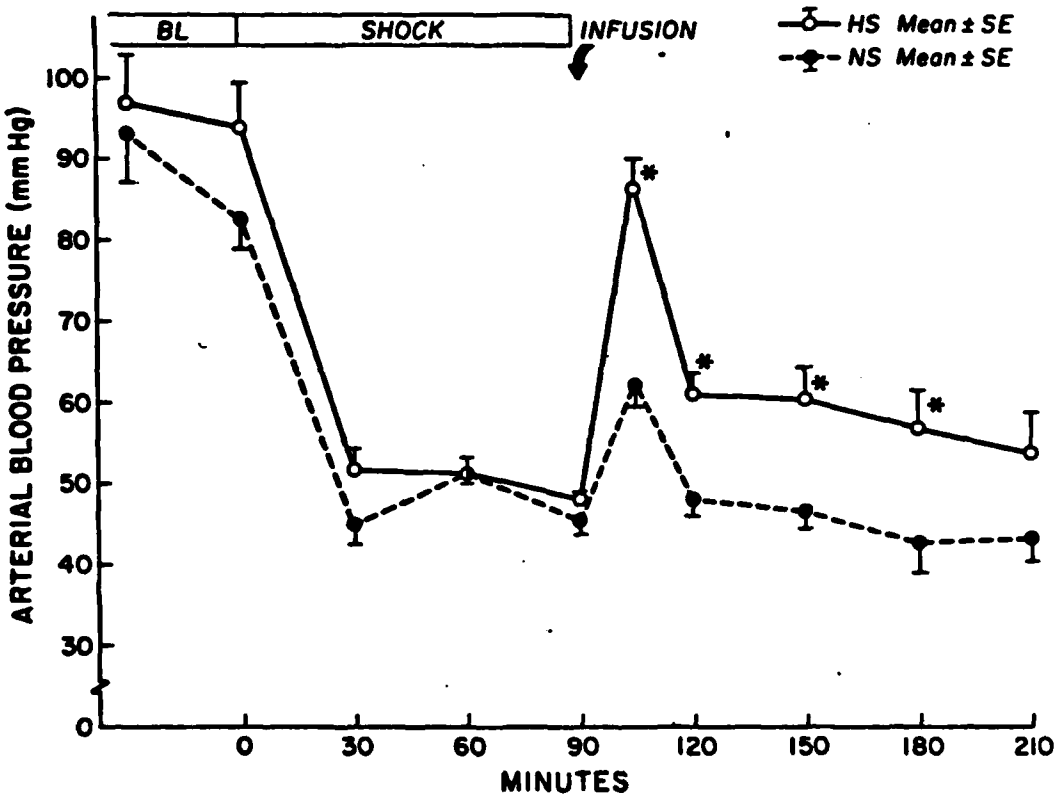

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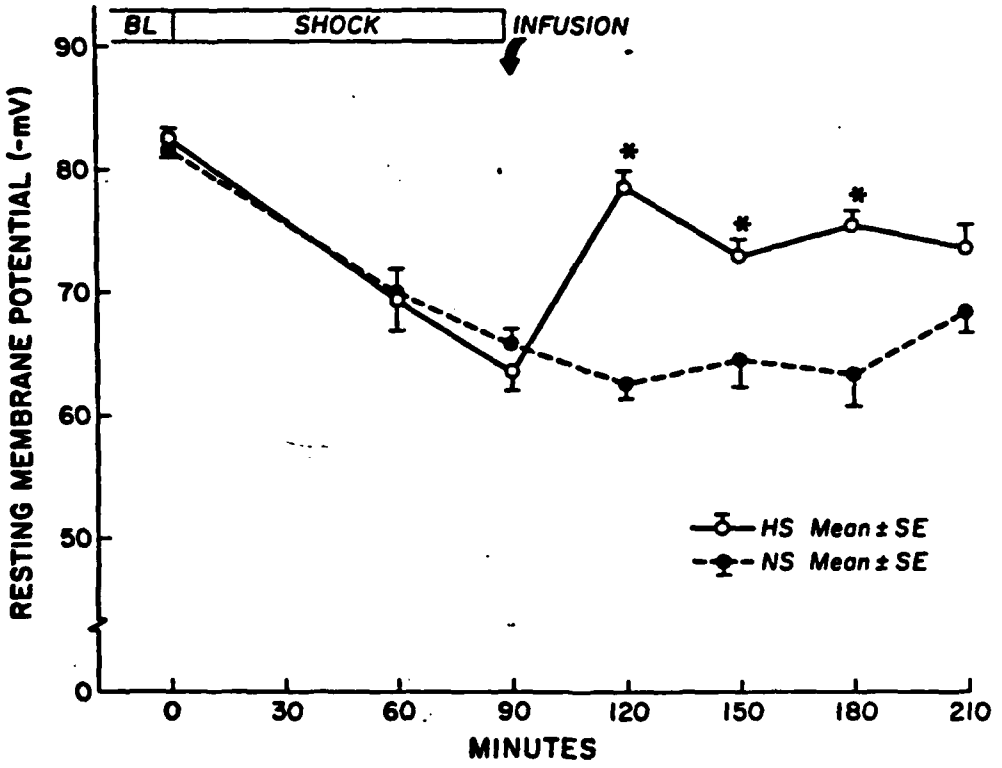

Figure 3: Resting skeletal muscle membrane potentials. See legend for Figure 2.

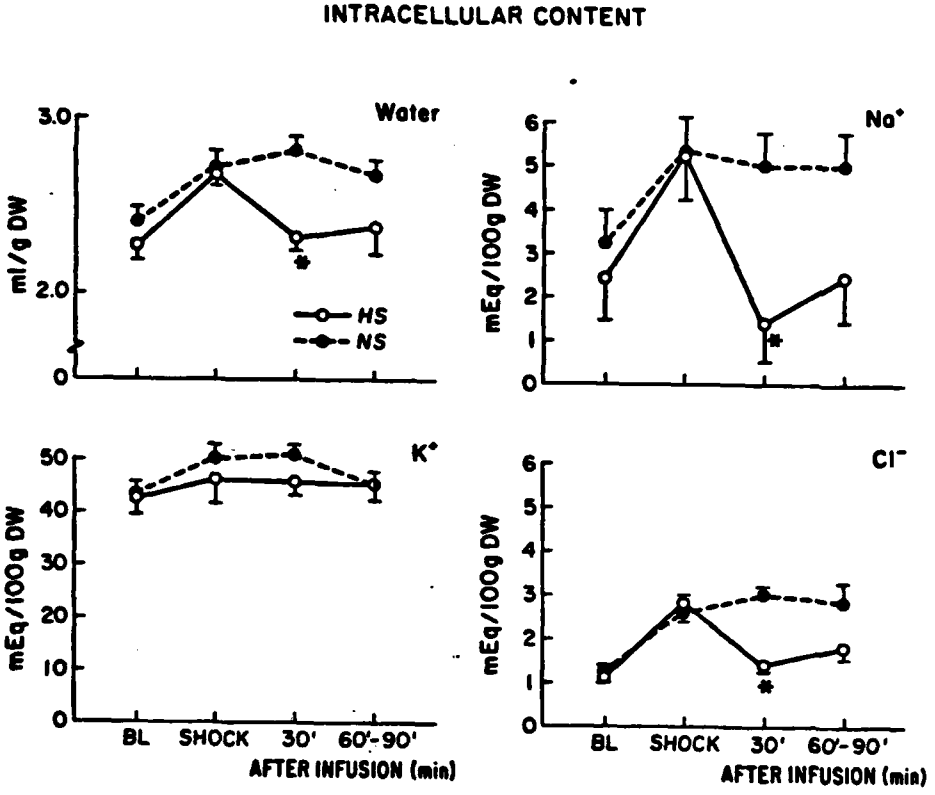

Figure 4: Intracellular contents of water, sodium, potassium, and chloride. See legend for Figure 2.

CONCLUSION AND FUTURE PLANS

Over the last year we have thus completed the studies that we had hoped to complete, as outlined in the initial contract proposal. We have also completed an additional study with measurements of skeletal muscle membrane potentials, intracellular water, and intracellular sodium and chloride concentrations.

We are now in the process of evaluating the very hypertonic sodium chloride solutions in models that will closely mimic clinical shock. These studies are progressing as initially outlined in our contract proposal.

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We also have evaluated small volume resuscitation with hypertonic sodium chloride and compared that resuscitation witn resuscitation using normal saline. The results of that study are indicated in the reprint on the following page. The hypertonic sodium chloride was effective for some 15-30 minutes after resuscitation was initiated. It was far more effective than using equivalent volumes of normal saline. Thus, the beneficial effects of the hypertonic sodium chloride solution rests in the solute concentration as opposed to the volume of the infusions used.

Circulatory Shock 13:149–159 (1984)

Small-Volume Resuscitation With Hypertonic Saline (2,400 mOsm/Liter) During Hemorrhagic Shock

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We compared small-volume resuscitation using either normal saline or hypertonic saline (2400 mOsm/liter) during hemorrhagic hypotension. Six unanesthetized sheep were bled to 50 mm Hg mean arterial pressure and maintained for 2 h. During this shock period cardiac output decreased 40–50% of baseline, while total peripheral resistance increased 20–30%. Then the response to a bolus injection of either hypertonic saline or normal saline, randomly chosen, was studied for an additional 2 h. The volume injected was 145–175 ml, equal to 10% of total shed blood volume. After data collection all shed blood was returned. Several days later, the protocol was repeated on each sheep with the alternate solution. After hypertonic saline the mean arterial pressure increased 48 mm Hg to 83% of control; with normal saline, mean arterial pressure increased 26 mm Hg. Cardiac output recovered to 95% of control immediately after infusion of hypertonic saline, while no significant increase was observed with normal saline. Ten minutes after injection of hypertonic saline, plasma volume increased ~360 ml, but with normal saline no increase was observed. We conclude that small-volume injection of hypertonic saline can dramatically improve circulatory function during hemorrhagic shock, as evidenced by expansion of plasma volume, increased cardiac output, and reduced peripheral resistance.

Key words: hemorrhage, hypertonic saline, hypotension, hypovolemia, osmolarity, resuscitation, sheep, shock

INTRODUCTION

Infusion of large volumes of isotonic salt solution is a highly successful and universally used initial treatment of hemorrhagic shock. Hyperosmolar solutions may be equally effective as a resuscitative fluid in experimental animals [1–4] and man [5–7]. Although both isotonic and hyperosmolar solutions can effectively restore

Submitted for publication September 3, 1983.

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vascular volume in shock, hyperosmolar resuscitation may have additional beneficial effects on the cardiovascular system. Hypertonic saline infusion increases myocardial contractility [1,8] and directly vasodilates the precapillary resistance vessels [9]. This vasodilatory effect apparently explains the reduced total peripheral resistance and associated increased mesenteric blood flow and oxygen uptake reported with hypertonic resuscitation [1, 8, 10].

Our laboratory has previously reported on a comparison of isotonic lactated Ringer's and hypertonic saline (600 mOsm/liter) infusions during hypovolemic shock in unanesthetized adult sheep [11]. We found that approximately 3 liters of either solution were required to return central venous pressure to its preshock value. The hypertonic resuscitation resulted in a significantly increased cardiac output coupled with reduced pulmonary vascular resistance.

A different approach to hyperosmolar resuscitation has been taken by Velasco et al [10], who found small volumes of very hypertonic saline (2,400 mOsm/liter) could successfully resuscitate anesthetized dogs from severe hemorrhagic shock. They reported that intravenous infusions of hypertonic saline equal in volume to only 10% of shed blood caused an immediate restoration of cardiac output, arterial pressure, and visceral blood flow. This improved circulatory function occurred without an increase in measured vascular volume and was sustained for 12 h without additional fluid therapy. From both a theoretical and practical viewpoint, these observations merit further examination.

The present report examines the effects of resuscitation with small volumes of either hypertonic saline (2,400 mOsm/liter) or normal saline in hypovolemic unanesthetized sheep. In each animal we produced two episodes of shock several days apart in order to effectively compare the two resuscitation regimens. It was our goal to determine if the dramatic and sustained effects reported in anesthetized dogs could be duplicated in unanesthetized sheep subjected to moderate hemorrhagic shock.

METHODS

Animal Preparations

Six adult female sheep, 40–50 kg, were anesthetized with halothane/nitrous oxide and surgically prepared with chronic cannulations of the thoracic aorta and superior vena cava using silastic catheters placed through a neck incision. A Swan-Ganz thermodilution catheter was placed to allow monitoring of central venous pressure, pulmonary artery pressure and cardiac output. Animals were kept unrestrained in cages and had free access to food and water until 36 h before an experiment, when both food and water were removed. The first experiment was performed 3–5 days after surgery. The day of an experiment a Foley catheter was inserted into the bladder for urine collection.

Measurements

Aortic, central venous, and pulmonary artery pressures were measured with Gould P23 Db pressure transducers and continuously recorded on a multichannel strip chart recorder. Transducers were leveled to the point of the shoulder. Cardiac output was measured using an Edwards Cardiac Output Computer. Blood gases were measured with an Instrumentation Laboratories Blood Gas Analyzer. Urine was collected in a closed drainage bag; volume was determined every 30 min using a graduated

cyclinder. Hematocrit was measured and plasma protein was determined with Biuret assay on arterial blood samples taken every 30 min. Serum sodium and potassium were measured on a Nova I Na^+/K^+ Analyzer. Plasma volume was measured with the dye dilution technique [12]. Intravenous injection of 10–20 mg Evans Blue (Harvey Labs) was followed by the sampling of arterial blood after 10, 20, and 30 min. Dye concentration in plasma was measured in a Gilford spectrophotometer. Regression analysis was used to determine dye concentration at time of injection. Plasma volume was calculated as dose injected divided by plasma concentration at time of injection. Total peripheral resistance (TPR, dyne·sec/cm^5) was calculated as the difference of mean arterial and central venous pressures (mm Hg); then this quantity was multiplied by 80 and divided by cardiac output (liter·min^{-1}). Minute work (N·m·min^{-1}) was calculated as mean arterial pressure multiplied by cardiac output multiplied by 1.33×10^{-4}.

Experimental Protocol

Experiments were performed on unanesthetized animals kept unrestrained in cages. After a 2–3-h control period of data collection, the sheep were bled to a mean arterial pressure (MAP) of 50 mm Hg through their venous canulae. All shed blood was stored in standard ACD blood bags (Fenwal Laboratories). The MAP was then maintained for 2 h at 50–60 mm Hg by further bleeding as required.

At the end of the 2-h shock period, animals were resuscitated by a bolus injection of either hypertonic saline, 2,400 mOsm/liter (HS), or normal saline (NS) and observed during the next 2 h. The response after injection was monitored for 2 h. The injected volumes of each resuscitative fluid was equal to 10% of total shed blood volume. Injections were given over 60 s and no subsequent resuscitative infusion followed.

After the 2-h postresuscitation period, all shed blood was returned. All animals recovered and then underwent a subsequent shock experiment 4–7 d after the first experiment using the same protocol except with the alternate solution. The identities of the resuscitative solutions were single blind coded and the sequence of solutions was determined with random-number tables.

Plasma volumes were measured during the preshock period, after 90 min of hypotension, and 10 min after resuscitation.

Statistical Analysis

Averaged values are expressed as mean ± standard error. One-way analysis of variance was used to determine if a variable changed with respect to time after resuscitation [13]. The paired Student t test was used to compare variable differences between the two solutions used on each sheep. Differences were considered significant when $P < .05$.

RESULTS

Mean hemodynamic and blood variables are shown in Tables I and II. Measured plasma volume during the baseline period was 1,853 ± 109 ml for the 12 experiments. Assuming an F cell ratio equal to 1.0 [14] we calculated baseline blood volume to be 2,657 ± 157 ml or 56.5 ml/kg.

TABLE I. Hemodynamic Changes During Hemorrhage and Small-Volume Resuscitation†

	Baseline period	Shock period (min)		Postresuscitation (min)			
		60	120	15	30	60	120
Normal saline treatment							
MAP*	108.0 ± 16.0	58.3 ± 5.7	57.8 ± 7.7	83.3 ± 13.8	76.3 ± 12.9	76.0 ± 14.0	72.0 ± 9.8
CVP	0.4 ± 0.6	−5.3 ± 1.0	−6.3 ± 1.0	−4.4 ± 1.0	−5.4 ± 1.0	−5.8 ± 1.0	−4.4 ± 0.9
HR	101.0 ± 12.8	88.0 ± 4.1	118.0 ± 14.1	153.0 ± 16.3	158.0 ± 22.0	147.0 ± 15.8	140.0 ± 13.7
Hypertonic saline treatment							
MAP*	123.3 ± 17.4	53.3 ± 4.5	53.3 ± 6.4	100.8 ± 15.0	107.5 ± 15.1**	85.0 ± 14.9	95.0 ± 14.3
CVP	0.6 ± 0.8	−3.3 ± 1.1	−5.5 ± 1.7	−1.5 ± 1.0**	−2.4 ± 1.0	———	−4.2 ± 0.8
HR	77.9 ± 11.2	83.3 ± 5.1	127.0 ± 19.3	182.0 ± 18.9	192.0 ± 13.8**	186.0 ± 12.4	177.0 ± 14.8

†MAP, mean arterial pressure in mm Hg; CVP, central venous pressure in mm Hg; HR, heart rate.
*$P < .05$ difference after injection, analysis of variance.
**$P < .05$ HS compared with NS, paired Student t test.

TABLE II. Blood Variables During Hemorrhage and Small-Volume Resuscitation†

		Baseline period	Shock period (min)		Post resuscitation (min)		
			60	120	30	60	120
Normal saline							
Hct	%	29.2 ± 1.2	27.3 ± 1.2	25.1 ± 2.0	24.3 ± 2.7	26.6 ± 2.9	26.3 ± 2.9
[P]	g/100 ml	7.7 ± 0.4	6.3 ± 0.3	5.3 ± 0.3	5.2 ± 0.3	5.1 ± 0.3	5.4 ± 0.4
Na+	mEq/liter	145.8 ± 1.4	143.4 ± 1.6	143.6 ± 1.8	144.1 ± 2.3	143.4 ± 2.1	143.1 ± 2.3
K+	mEq/liter	3.9 ± 0.1	4.2 ± 0.3	4.1 ± 0.2	3.9 ± 0.4	3.7 ± 0.3	3.6 ± 0.2
Hypertonic saline							
Hct	%	31.1 ± 1.1	26.7 ± 1.1	26.6 ± 1.3	23.3 ± 1.1	24.1 ± 1.2	23.8 ± 1.5
[P]	g/100 ml	7.4 ± 0.4	6.1 ± 0.4	5.6 ± 0.4	4.9 ± 0.3	5.2 ± 0.3	5.2 ± 0.3
Na+*	mEq/liter	144.3 ± 1.2	142.3 ± 1.5	143.9 ± 1.5	153.5 ± 1.9**	151.0 ± 2.0**	151.0 ± 1.5**
K+*	mEq/liter	3.9 ± 0.1	4.3 ± 0.2	3.7 ± 0.1	2.8 ± 0.2**	3.1 ± 0.1	3.3 ± 0.1

†[P], plasma protein concentration, g/100ml.
*$P < .05$ difference after injection, analysis of variance.
**$P < .05$ HS compared with NS, paired Student t test.

Shock Period

No significant differences were found between the two paired experiments during the shock period. Mean arterial blood pressure was lowered to 50 mm Hg within 30 min with an initial removal of 1,100–1,300 ml of blood. After blood pressure fell below 60–70 mm Hg each animal would lie down in its cage. The animals experienced no apparent pain during the hypotension and were generally lethargic but conscious for the entire shock period. Although arterial pO_2 did not deviate from its baseline levels (85–95 mm Hg), respiratory rate invariably increased three to four times during hypotension.

Maintenance of blood pressure at 50–60 mm Hg required the removal of an additional 300–500 ml blood over the last 90 min of the shock period. Average shed blood volume was 35 ml/kg or 62% of initial blood volume. Plasma volume measured at 90 min into the shock period was 1,520 ml (Fig. 1); calculated blood volume was 2,051 ml. Comparison of preshock and shock blood volumes with shed blood indicates that over 60% of the volume of blood loss had been replaced by an autotransfusion of extravascular fluid and cells.

During the shock period central venous pressure remained 5–7 mm Hg below baseline (Table I); pulmonary artery pressure decreased in all animals from a mean baseline value of 15.0 to 10.8 mm Hg. After blood pressure had been reduced to 50 mm Hg, cardiac output decreased to 40–50% of baseline and remained at this level during the remainder of the shock period (Fig. 2).

Resuscitation

After each animal received a bolus injection of either hypertonic saline (HS), or normal saline (NS), there was an immediate increase in mean arterial pressure (Table I). Within 2 min after injection of HS arterial pressure increased 48 mm Hg and mean pulse pressure increased to 60 mm Hg. After NS the mean arterial pressure was

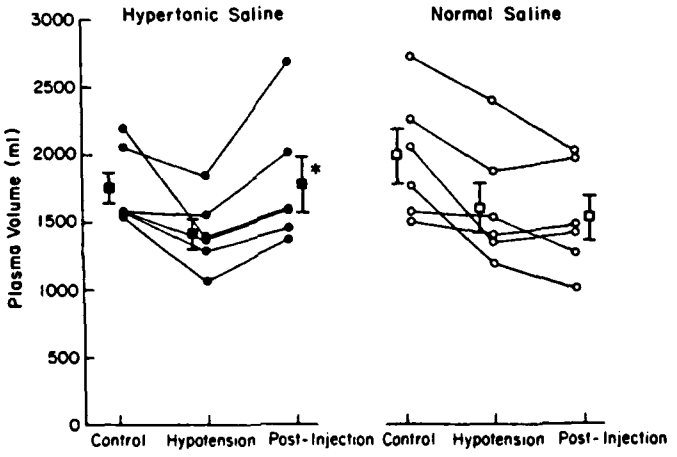

Fig. 1. Changes of plasma volume during shock and after resuscitation in both groups are shown. Plasma volume was significantly increased after hypertonic saline to a level similar to its baseline value. After normal saline, mean plasma volume remained similar to its shock value. $^*P < .05$, plasma volume increased after hypertonic saline.

$P > .10$ difference after injection, analysis of variance.
$^{**}P < .05$ HS compared with NS, paired Student t test.

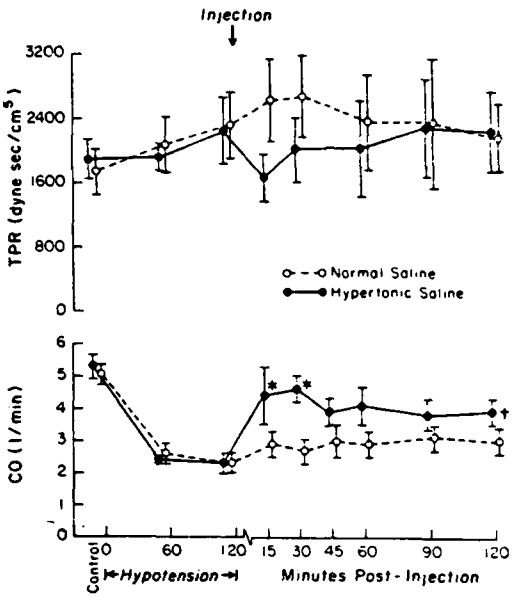

Fig. 2. Effects of hemorrhagic shock and resuscitation on cardiac output (CO) and total peripheral resistance (TPR) are shown. CO was increased significantly at 15 and 30 min after injection of hypertonic saline. TPR reduced transiently after administration of hypertonic saline. TPR after the injection of normal saline continued to increase. Means ± 1 SE. $^{*}P < .05$, hypertonic saline compared with normal saline. $^{+}P < .05$, increased CO after hypertonic saline injection.

increased only 26 mm Hg and the average pulse pressure was equal to 28 mm Hg, which was significantly smaller than with HS.

Cardiac output also rapidly increased after injection of HS (Fig. 2). At 15 and 30 min post HS injection cardiac output was 88–96% of its baseline value compared to 49–58% in the NS experiments. Total peripheral resistance was reduced after HS injection, while with NS mean peripheral resistance continued to increase (Fig. 2). However, during the second hour after injection neither cardiac output nor total peripheral resistance was significantly different between the two protocols.

Stroke volume did not change significantly with injection of HS (Fig. 3). Minute work was significantly increased after injection of HS (Fig. 3).

Plasma volume measured 10 min after injection was significantly increased after HS to a level similar to its baseline value; after NS plasma volume was less than during the shock phase (Fig. 1). The mean injection volume of HS was 162 ml and caused an increase in average plasma volume of 362 ml compared to the shock period. After injection of 168 ml of NS the measured plasma volume was unchanged from its shock value. Plasma protein concentrations [P] (Table II) were similar with both protocols at all times.

Plasma concentrations of sodium and potassium were unaffected by shock and injection of NS (Table II). On the other hand, injection of HS increased plasma sodium 7% to 154 mEq/liter and decreased potassium from 3.7 to 2.8 mEq/liter.

Urinary output fell to 17–21% of baseline during the second hour of shock but was increased after injection with both protocols (Fig. 4). The HS resulted in a

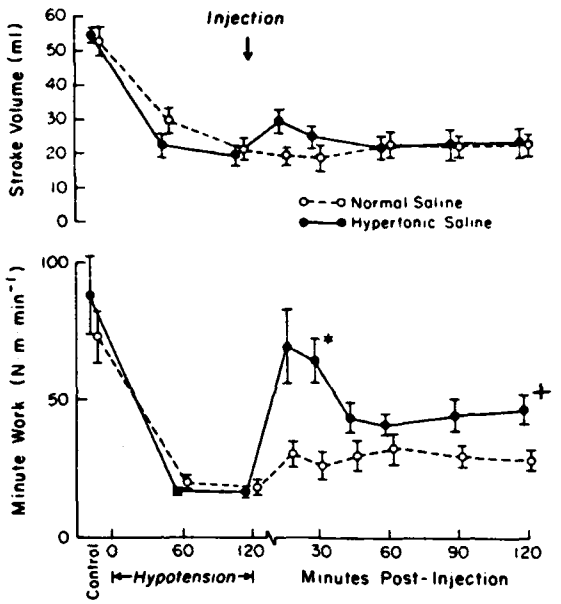

Fig. 3. Effects of hypotension and resuscitation on stroke volumes and cardiac minute work are shown. Minute work ($N \cdot m \cdot min^{-1}$) was calculated as MAP × CO × 1.33 × 10^{-4}. There was no apparent change in stroke volume after hypertonic saline injection. $^{*}P < .05$, hypertonic saline compared with normal saline; $^{+}P < .05$, increased cardiac work after hypertonic saline.

comparatively large output, three times baseline, during the first hour postinjection. During the same hour urine output after NS injection was less than half baseline.

DISCUSSION

We have found that the chronically catheterized unanesthetized sheep is an excellent preparation in which to study hypovolemic shock and fluid resuscitation. The level of shock induced was clinically relevant: a blood loss of more than 60% of initial vascular volume, a fall in arterial pressure to 50–60 mm Hg, and a cardiac output of less than half baseline. This moderate level of shock was completely reversible in the unanesthetized animal and allowed us to compare two resuscitation regimens during separate hypovolemic periods in each animal. The physiological responses to the two shock periods separated by a period of 4–7 d were virtually identical.

In preliminary experiments we found that a similar shock protocol in anesthetized sheep invariably resulted in death within 2 h. The striking difference in survival time between anesthetized and unanesthetized animals underlines the effectiveness of the physiological reserves that the conscious animal can initiate.

Our objective was to determine the effectiveness of small-volume infusion of very hypertonic saline in the initial resuscitation of hypovolemic shock. A recent study [10] suggested that infusion of a small volume of hypertonic saline (2,400 mOsm/liter) can permanently reestablish normal cardiovascular function during se-

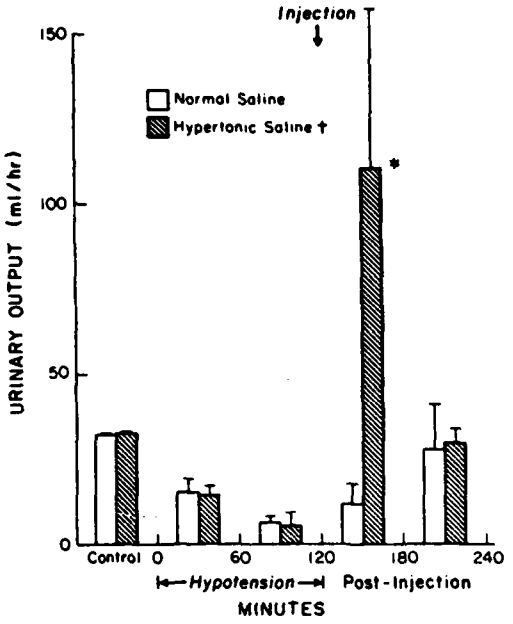

Fig. 4. Effects of resuscitation on urinary output are shown. Urinary output after hypertonic saline is markedly increased. During the first hour after injection of hypertonic saline urine flow rate was three times baseline. $*P < .05$, hypertonic saline compared with normal saline; $^{\dagger}P < .05$, increased urinary output after hypertonic saline.

vere hemorrhagic shock. Other studies indicate that hypertonic saline can effectively be used for volume replacement, but the observed beneficial effects were described as transitory [8,15]. Specifically we wanted to monitor cardiovascular dynamics after small-volume resuscitation and determine if the observed beneficial effects were sustained.

Shock Period

Significant physiological reserve was demonstrated by unanesthetized sheep in response to removal of an average of 1.6 liters of blood. Ninety minutes into the shock period 69.8% of the removed plasma and 45.6% of the red cell deficit had been replaced by autotransfusion. This replenishment of vascular volume probably resulted from a combination of the following factors: the sheep's contracting spleen [16], capillary refill due to a lowered capillary pressure [17], a glucose-induced hyperosmolality [4, 18], and increased peripheral lymph flow [11, 19]. The extensive autotransfusion necessitated frequent withdrawal of blood during the entire shock period to prevent a significant return of blood pressure and cardiac output. In preliminary experiments we found that in order to induce a reproducible level of shock, animals had to be deprived of water for 36 h before each experiment.

Fluid Resuscitation

In a previous investigation [11] we reported that an average of 3.2 liters of isotonic lactated Ringer was required to return and maintain central venous pressure in adult sheep after 2 h of hypovolemic shock. This amount of fluid, slightly over twice the volume of shed blood, is representative of volumes used in current clinical resuscitation regimens. In the present study, we examined the effects of resuscitation with a fluid volume equal to one twentieth the volume used in our previous studies. Injection of 168 ml of normal saline significantly increased arterial pressure from 57.8 to 83.3 mm Hg, but small changes were observed in cardiac output or central venous pressure. By contrast, the most apparent effect of hypertonic saline was the increased cardiac output, which returned to 96% of its preshock value. Hypertonic saline injection also caused significant increases in both arterial pressure and central venous pressure, and caused a fall in total peripheral resistance.

Undoubtedly, some of these changes are due to the extracellular volume expansion caused by infusion of an osmotic load. We can estimate normal intracellular water = 40% of body weight, extracellular water = 20%, hematocrit = 30%, and osmolality = 300 mOsm/liter. After a blood loss of 1,600 ml in a 45-kg sheep and infusion of 160 ml of hypertonic saline, we calculate that ~710 ml of water moves from cells into the extracellular space. Assuming that the 870 ml expansion (160 + 710) is distributed between the blood and interstitium in a 1:2 ratio we predict an expansion of plasma volume of ~300 ml. This is in reasonable agreement with the measured increase in average plasma volume of 360 ml. Plasma volume generally continued to decline with normal saline. The average difference in plasma volume expansion between the two protocols was 255 ± 118 ml.

The decrease in cellular water, estimated to be about 4%, did not cause any observed deleterious effects. Obviously, there is a limit to the amount of hypertonic saline that can be safely infused, but 3–4 ml/kg appears safe based on our studies in sheep and the studies of Velasco et al in dogs [10]. The rise in serum sodium to 153 mEq/liter is not as alarming as the observed fall in serum potassium from 3.7 to 2.8 mEq/liter (Table II). This rapid 24% decrease in potassium concentration cannot be explained by expansion of the extracellular space, estimated to be = 10%. Neither can it be explained by renal loss, which is insignificant at 10 min after injection. Further research is required to define the mechanisms and dangers of electrolyte changes subsequent to resuscitation with very hypertonic saline.

Another clear difference between the two protocols was the fall in total peripheral resistance after hypertonic saline, while peripheral resistance remained unchanged with normal saline. Although this decrease in total peripheral resistance results partly from the expanded vascular volume, direct vasodilatory action of hyperosmolality may also contribute. Direct vasodilatory effects of hypertonicity have been well documented [9, 20] and may help explain the increased cardiac output in our study as well as improved peripheral blood flow reported in other studies [8, 11].

Hypertonicity may directly improve cardiac performance by increasing contractility and cardiac efficiency [1, 8, 21–23]. We found that hypertonic saline increased heart rate when compared to normal saline despite a higher arterial pressure with hypertonic saline. Baroreceptor feedback should be greater in the hypertonic group because of the higher arterial pressure; thus the greater heart rate is not easily

explained. A direct chronotropic effect may be beneficial but in a compromised myocardium the increased oxygen consumption could be deleterious. Minute work [24] was significantly increased through the first 30 min after hypertonic infusion. Heart rate and cardiac output increased the same relative amounts as there was no apparent change in stroke volume (Fig. 3). This contrasts somewhat with other studies in which stroke volume was increased [1, 10] after hypertonic saline infusion.

Another consistently observed effect of hypertonic saline was diuresis: Urine output was at least three times baseline during the first hour after HS injection. Glomerular filtration rate may have increased secondary to improved renal perfusion, but we have no direct evidence to support this. This apparently inappropriate diuresis during hypovolemia was surprising since hypertonic saline infusions have been shown to cause release of anti-diuretic hormone [25]. Hypertonic saline treatment could prove advantageous if it increases renal blood flow and urinary output during shock-induced renal insufficiency.

Although our study clearly showed that a small volume injection of hypertonic saline caused rapid and significant improvement in cardiovascular function, the improvement was not permanent. Arterial pressure and cardiac output both decreased from their early post injection levels. During the second hour after injection there was not a statistically significant difference between the two solutions for either cardiac output or arterial pressure although averaged values remained greater in the hypertonic saline group.

Despite the lack of a permanent effect, small-volume resuscitation could offer important advantages as an initial therapy. Rapid mobilization of cellular water and other direct cardiac and vascular effects of hypertonicity might return cardiovascular function more rapidly than traditional resuscitation. Thus, hypertonic saline treatment could prove beneficial as the initial treatment of shock and could be followed by a larger volume of isotonic fluid as needed.

ACKNOWLEDGMENTS

We wish to thank Ted Wandzilak for his excellent technical assistance and Mrs. Judy Hendrickson and Mrs. Phoebe Ling for preparing the manuscript. This work was supported by Department of Defense Contract No. DAMD 17-83-C-3175. Dr. Nakayama is supported by a fellowship from the Ministry of Health and Welfare, Japan. Dr. Kramer is a pulmonary fellow of the Francis B. Parker Foundation.

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In conducting the research described in this report the investigators adhered to the NIH guidelines for the use of experimental animals.

We have also evaluated the use of very hypertonic sodium chloride in rats that have been subjected to moderate hemorrhagic shock. The manuscript for that study is on the next page. Infusion of very hypertonic sodium chloride restores resting skeletal muscle transmembrane potentials to normal. It also mobilizes water out of the intracellular space and into the extracellular space. Curiously, infusion of very hypertonic sodium chloride also allows the cell to maintain normal sodium and chloride concentrations intracellularly.

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